

# Photosynthesis and Environmental Stress

## Interactions in Sugarbeet Leaves\*

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### INTRODUCTION

One of the greatest needs in both research and practical crop production is a method for measuring instantaneous plant growth. If we could go out in the field and make simple rapid measurements of plant growth rates, the application and benefits would be immediate and far reaching. The instantaneous growth rate is characterized by the carbon balance of a plant or crop. Some years ago Terry (9) and some of his associates made detailed studies of CO<sub>2</sub> exchange parameters of beet leaves effected by nutrient deficiencies. Under carefully controlled conditions he found changes that developed in the very early stages of nutrient stress. Following this lead I attempted to make a practical application on sugarbeets (*Beta vulgaris* L.) growing under real field conditions (1). The objective was not accomplished because variation in CO<sub>2</sub> exchange was greater from leaf to leaf than the changes brought on by the initiation of stress.

The data and results reported here come from additional studies using field-grown sugarbeet leaves. The objective was to pinpoint the fundamental differences in the leaves that lead to the large variability in CO<sub>2</sub> assimilation from leaf to leaf.

Since these differences may be selectively effected by various types of plant stress, nitrogen and water variables were applied on the filed plots. Temperatures were also monitored with particular attention to the cool periods that occurred.

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Four steps occur during the course of  $\text{CO}_2$  fixation, any of which can individually limit the rate of assimilation.

1. The  $\text{CO}_2$  must diffuse through the stomata into the leaf interior.
2. The  $\text{CO}_2$  must diffuse through the gas phase of the mesophyll tissue to the cells with chloroplasts.
3. Transport of  $\text{CO}_2$  in the liquid phase through cell walls into the chloroplasts must occur.
4. A sufficient supply of both RuBP (ribulose-1,5 biphosphate) and active carboxylating enzyme sites must be present in the chloroplasts.

These four factors may be expressed as individual resistances to  $\text{CO}_2$  fixation. The size of each resistance may be estimated from gas exchange measurements made under carefully controlled conditions. A fifth factor, leaf respiration, must also be considered in this type of analysis, for when the four resistances are low allowing rapid  $\text{CO}_2$  fixation, a high respiration rate can negate the net result.

Calculation of the values of these five factors was made for individual leaves. Steps in the  $\text{CO}_2$  assimilation path that led to the large differences in photosynthesis among field grown sugarbeet leaves are discussed in light of the results.

#### MATERIALS AND METHODS

Sugarbeets were grown in the field in Southcentral Idaho on the portneuf silt loam soil ( *Durixerollic calciorthid* ) which has been described in detail (2). The control treatment was fertilized with N and P and irrigated from furrows in accord with prevailing best management recommendations. Other areas were managed to create either N or water stress by not fertilizing and discontinuing irrigation after July 7.

Throughout the growing season leaves were removed with their petioles submerged in water and brought into the laboratory for detailed gas exchange measurements in the chamber described previously (3). Gas exchange measure-

ments were made using both sides of the leaf with a flow rate of  $1\frac{1}{2} \text{ min}^{-1}$  over  $24.5 \text{ cm}^2$  of leaf surface except during August when a connection was inadvertently plugged during repairs causing the gas to flow only across the underside of the leaf surface. Tests showed this reduced net  $\text{CO}_2$  assimilation by 15 or 20 percent, but had little effect on the relative differences between the resistances being studied.

Measurements of  $\text{CO}_2$  diffusion resistance in the gas phase of the mesophyll tissue were made by varying the ambient pressure and applying the analysis developed previously (3). The assimilation of  $\text{CO}_2$  was measured at two light intensities, 715 and  $260 \mu\text{e m}^{-2}\text{s}^{-1}$ , and at least three ambient  $\text{CO}_2$  levels ranging from 220 to  $800 \text{ mg m}^{-3}$ . Values of the physical part of the liquid phase mesophyll  $\text{CO}_2$  transfer resistance across the cell walls and membranes were calculated from linear regression of the  $\text{CO}_2$  response curves using Jones and Slatyer's equation with ambient  $\text{O}_2$  held at one percent (7). The stomatal and cuticular resistance,  $r_s$ , was obtained from transpiration rates and included  $110 \text{ s m}^{-1}$  of boundary layer resistance resulting from the leaf chamber geometry and the gas flow rate (3).

## RESULTS

A total of 63 leaves were taken into the laboratory and their  $\text{CO}_2$  exchange properties studied in detail. Typical results including the more interesting data are presented in Table 1. The table includes four sections with the leaves in each section ranked according to the amount of carbon they were able to fix under high light and  $\text{CO}_2$  conditions. The measurements made on these leaves were not intended to mimic photosynthetic rates in the field, but rather to search for differences in gas exchange parameters that may have been imprinted by previous conditions as the leaves grew in the field.

The dark respiration was measured the first minute or so after the light was turned off. In general the values were little different, falling in the range of 0.06 to

Table 1. Net  $\text{CO}_2$  fixation rates of field grown sugarbeet leaves as affected by a variety of conditions. The symbols are defined as H, high light  $715 \mu\text{e m}^{-2}\text{s}^{-1}$ ; L, low light  $260 \mu\text{e m}^{-2}\text{s}^{-1}$ ; h, high ambient  $\text{CO}_2$  concentrations of  $800 \text{ mg m}^{-3}$ ; l, low  $\text{CO}_2$  at  $220 \text{ mg m}^{-3}$ ; P net  $\text{CO}_2$  uptake  $\text{mg s}^{-1}\text{m}^{-2}$ ;  $r_s$ , stomatal and boundary resistance to  $\text{CO}_2$  diffusion  $\text{s m}^{-1}$ ;  $r$ ,  $\text{CO}_2$  resistance through the mesophyll cell walls and membranes into the chloroplasts,  $\text{s m}^{-1}$ ; and  $r_c$  apparent carboxylation resistance,  $\text{s m}^{-1}$ .

Leaf	Date	Light	$\text{CO}_2$	P	$r_s$	r	$r_c$	Leaf Description
Leaves from the control treatment								
1	09-13 PM	H H	h l	1.08 0.43	190 160	249	281 53	Young, large sink, optimum conditions
2	06-27 PM	H H L L	h l h l	0.92 0.27 0.49 0.27	330 450 450 290	211 474	315 60 764 37	Expanding, optimum conditions
3	07-16 AM	H H	h l	0.83 0.43	390 570	193	425 177	Mature, a bit lighter green but N fertilized
4	08-16 AM	H H	h l	0.70 0.29	230 250	564	378 (-66)	Expanding, optimum conditions
5	08-15 PM	H H	h l	0.69 0.38	240 180	313	604 111	Mature, partly shaded
Leaves from nitrogen stressed plants								
6	08-13 PM	H H	h l	0.92 0.45	200 200	217	417 28	Expanding, a bit chlorotic
7	07-18 PM	H H	h l	0.81 0.37	320 240	192	514 138	Mature, light green

Table 1 - Continued

Leaf	Date	Light	CO <sub>2</sub>	P	r <sub>g</sub>	r	r <sub>c</sub>	Leaf Description
8	08-28 PM	H H L L	h l h l	0.81 0.24 0.30 .022	340 350 1020 360	517 435	116 2 1244 132	Expanding, light green
9	09-13 AM	H H	h l	0.64 0.36	190 210	338	432 23	Young, still dark green
10	09-11 AM	H H	h l	0.29 0.10	310 550	1343	976 457	Mature, somewhat chlorotic
Leaves from water stressed plants								
11	08-21 AM	H H	h l	0.98 0.27	240 320	267	314 168	Mature, flaccid on the previous afternoon
12	08-28 AM	H H	h l	0.79 0.19	480 540	552	(-14) 43	Expanded, flaccid on the previous afternoon
13	09-11 PM	H H L L	h l h l	0.49 0.19 0.41 0.11	510 320 390 360	830 3175	268 (-116) (-1573) (-1539)	Mature, flaccid on the previous afternoon
14	08-30 PM	H H	h l	0.48 0.16	830 520	957	(-127) (-150)	Expanded, a bit flaccid
Leaves from cold stressed plants in the control treatment								
15	08-20 PM	H H	h l	0.73 0.16	470 560	1087 1087	(-453) (-339)	Expanded, weather unseasonably cool

Table 1 - Continued

Leaf	Date	Light	CO <sub>2</sub>	P	r <sub>s</sub>	r	r <sub>c</sub>	Leaf Description
16	08-22 PM	H H	h l	0.73 0.14	430 460	1010	(-308) (-469)	Expanded, weather unseasonably cool
17	09-10 AM	H H	h l	0.59 0.31	210 240	375	743 21	Expanding, cold following warm
18	11-02 AM	H H	h l	0.59 0.11	290 420	299	382 721	Expanding, freeze hardy
19	11-01 AM	H H	h l	0.12 0.10	1140 870	2345	4200 740	Expanding, not freeze hardy

0.08 mg s<sup>-1</sup> m<sup>-2</sup>. Younger leaves tended to show larger values as did several of the cold and water stressed plants. Leaf 1 had highest respiration rate of 0.19. It was from a plant in a group in the optimum fertility and water area that had all leaves removed the third week in August. The young leaves that grew from these plants showed high rates of CO<sub>2</sub> fixation and large initial dark respiration. This may have been associated with their large root to leaf ratios providing unusually large sinks for the newly fixed carbon.

The resistances to CO<sub>2</sub> diffusion in the gas phase of the mesophyll tissue did not show any striking differences due to the treatments, falling generally in a range less than 200 m s<sup>-1</sup>.

The measurements at low light levels were not as interesting as those at saturating light because the experimental errors are larger by comparison than those at high light where the CO<sub>2</sub> flux is greater. The results at low light did show the same general trends at those under high light, though the resistances were generally larger. Results from low light observations are included in Table 1 for leaves 2, 8, and 13, to illustrate the range of numbers that occurred.

The values for the resistance to CO<sub>2</sub> transport from the cell wall into the chloroplast,  $r$ , and the residual "carboxylation" resistance,  $r_c$ , are the most interesting parameters. Values for  $r_c$  were calculated from the classical analogue resistance relation, which defines  $r_c$  as the apparent residual, i.e.,

$$P = \frac{C_a - C}{r_s + r} = \frac{C}{r_c} \quad (1)$$

where  $P$  is the gross rate of CO<sub>2</sub> fixation,  $C_a$  the concentration of CO<sub>2</sub> in the air outside the leaf,  $r_s$  the combined stomatal and boundary resistance to CO<sub>2</sub> transport, and  $C$  is the average concentration of CO<sub>2</sub> in the chloroplasts. Assuming second order kinetics one may also express  $P$  as

$$P = kC[E-RuBP] \quad (2)$$

where  $k$  is the rate constant and  $[E-RuBP]$  is the concentration of RuBP attached to active carboxylating enzyme sites and thus ready to react with  $CO_2$  to form PGA. Combining eqs. (1) and (2) to eliminate P/C gives

$$r_c = \frac{1}{k} \frac{1}{[E-RuBP]}. \quad (3)$$

While values of  $r_c$  are calculated from experimental data with eq. 1, the values are more meaningful when considered in terms of eq. 3 which shows that values of  $r_c$  are inversely proportional to the concentration of RuBP adsorbed on active carboxylating sites in the chloroplasts. Thus, when values of  $r_c$  are large, it follows that the concentration of RuBP and/or the activity of the carboxylating sites in the chloroplasts are low and may limit photosynthesis.

At high levels of ambient  $CO_2$ ,  $r_c$  becomes large because the amount of  $CO_2$  in the chloroplasts begins to saturate the reaction making the number of active carboxylating sites or the amount of RuBP the limiting factor. On the other hand, under low  $CO_2$  the carboxylating resistance may be low because carboxylase activity and RuBP is high with respect to the amount of  $CO_2$  in the chloroplasts, eq. 2. When  $r_c$  remains large at low  $CO_2$  values the inherent ability of the chloroplasts to fix carbon is surely impaired.

As the rate of carbon fixation decreases the results in Table 1 show that either the liquid phase transfer resistance to  $CO_2$  flow into the chloroplasts increases, or the carboxylation resistance increases, or both. For example, leaf 4 fixed less carbon than leaf 2 because of a larger resistance to  $CO_2$  transport into the chloroplasts though there was no obvious reason why this should have occurred. Leaf 5 fixed less carbon than 3 because of higher resistances to both  $CO_2$  transport into the chloroplast and to carboxylation. It may be noted that the experimental error involved in measuring the  $CO_2$  assimilation rates was no more than  $\pm 0.03 \text{ mg m}^{-2}\text{s}^{-1}$ . Thus the difference was real in the leaves' abilities to fix  $CO_2$ .



at high light and ambient  $\text{CO}_2$  levels.

Nitrogen deficient leaves that showed the higher rates of  $\text{CO}_2$  assimilation tended to show some limitations due to carboxylating activity, i.e., leaves 6 and 7, compared to 1 and 2. At lower rates however, liquid phase transport resistances were large. Water stressed leaves fixing carbon at lower rates did show predominantly high resistances to  $\text{CO}_2$  transfer into the chloroplasts (leaves 12-14). This has also been observed in water stressed cotton leaves (6). In leaves 12-14, resistances were so large they forced negative values for  $r_c$  which is not in keeping with the physical model on which  $r$  and  $r_c$  are based. The same problem is evident for leaves 15 and 16. The weather had been unseasonably cool, over cast and damp for several days beginning August 17. After being conditioned to this type of weather in the field sugarbeets may typically show some wilting on the first warm day with full sun, even though soil water is adequate; so perhaps the large values of  $r$  for leaves 15 and 16 resulted from the same phenomena manifest in leaves 12-14. Other leaves studied during the period August 18-24 that were from low nitrogen or soil water areas did not show the high  $r$  values, for example, leaf 11.

Leaf 17 exhibited a different type of cold response than leaves 15 and 16. In this case the weather had been unseasonably warm for several days, maximum over  $30^\circ\text{C}$  and minimum only  $11^\circ\text{C}$ . On the 10th of September the low was  $7^\circ$  and the high  $23^\circ\text{C}$ . That afternoon analysis of leaf 17 showed an unusually high carboxylating resistance. High carboxylating resistances following leaf desiccation had been reported (4), but other studies may be cited suggesting the carboxylating system can recover during prolonged water stress (4).

Measurements were continued into early November after the leaves had been freezing for several nights with lows of  $-4^\circ\text{C}$ . While the leaves thawed and looked healthy during the day their stomata were sluggish and slow to open in the mornings. Leaves 18 and 19 visually appeared

to be identical but leaf 19 had obviously been injured by freezing for its stomata resisted opening even in the laboratory. Its  $r$  and  $r_c$  values, as well as its respiration, were large. The leaf was from one of the plants that had been defoliated in August while leaf 18 was from a nearby plant that had not had its leaves removed.

#### DISCUSSION AND CONCLUSION

My confidence in the values of  $r$  is no more than  $\pm 100$  s  $m^{-1}$  based on variation of the measurements that made up the  $CO_2$  response curves. The values were obviously too large when they forced  $r_c$  to be negative. This is a serious limitation in the analysis of stepwise resistances to  $CO_2$  assimilation. It probably arises from some of the assumptions made in the Jones and Slatyer derivation which are not adequate for all types of stressed leaves grown in the field, i.e.,  $r$  may sometimes be a function of internal leaf  $CO_2$  levels.

One may ask what effect removing the leaf from the plant has on  $CO_2$  responses. In the case of sugarbeets, removal evidently does not cause much change for several hours provided the petiole is kept submerged in water. In preliminary trials, leaves attached to potted plants were placed in the chamber and allowed to come to steady state under the high light and  $CO_2$  conditions. The petioles were then cut and after a minute or so the  $CO_2$  assimilation returned to its previous steady level and remained near there for about five hours before beginning to gradually decrease. The data reported here were obtained within at least 3.5 hours following leaf removal.

While the experiment described here was of an exploratory nature, the four types of resistances did not generally indicate sharp individual correlations with differently stressed leaves that had obviously developed different inherent abilities to fix  $CO_2$ . The inherent differences themselves did seem to transcend the change from field plants to excised leaves fixing  $CO_2$  under controlled laboratory conditions. In general, the data indicated that the resistance to  $CO_2$  transport from the cell wall

into the chloroplast may increase following water stress and some types of chilling. In other cases, the  $\text{CO}_2$  fixation rate is limited more by the carboxylation resistance which maybe interrupted as less than optimum amounts of RuBP attached to active carboxylation sites in the chloroplasts. Differences in stomatal resistance, gas phase mesophyll resistance, and dark respiration were generally small.

Unfortunately, it is still not apparent how one might make a simple  $\text{CO}_2$  exchange measurement on a few leaves in the field that would signal the onset of plant stress before any visual signs occur. We must better understand and model the kinetics of  $\text{CO}_2$  assimilation to reach that important goal. Nevertheless, it is obvious that differences do exist and, because they do, the potential for progress is real.

#### LITERATURE CITED

- (1) Cary, J. W. 1977. Photosynthesis of sugarbeets under N and P stress: Field measurements and carbon balance. *Agron. J.* 69:739-744.
- (2) Cary, J. W., and W. W. Rasmussen. 1979. Response of three irrigated crops to deep tillage of a semi-arid silt loam. *Soil Sci. Soc. of Am. J.* 43:574-577.
- (3) Cary, J. W. 1981. Calculation of  $\text{CO}_2$  gas phase diffusion in leaves and its relation to stomatal resistance. *Photosynthesis Research* 2:185-194.
- (4) Heuer, B., and Z. Plaut. 1981. Carbon dioxide fixation and ribulose-1, 5-biphosphate carboxylase activity in intact leaves of sugarbeet plants exposed to salinity and water stress. *Ann. Bot.* 48:261-268.
- (5) Hodanova, D. 1979. Sugarbeet canopy photosynthesis as limited by leaf age and irradiance: Estimation by models. *Photosynthetica* 13:376-385.
- (6) Jones, H. G. 1973. Moderate term water stress and associated changes in some photosynthetic parameters in cotton. *New Phytol.* 72:1095-1105.
- (7) Jones, H. G. and Slatyer, R. O. 1972. Estimation of the transport and carboxylation components of intracellular limitation to leaf photosynthesis. *Plant Physiol.* 50:283-287.

- (8) Loomis, R. S., and E. Ng. 1977. Influences of climate on photosynthetic productivity of sugarbeet. Proc. 4th Int. Congr. on Photosynthesis, p. 259-268. Printed in Great Britian.
- (9) Terry, N., and A. Ulrich. 1973. Effects of phosphorus deficiency on the photosynthesis and respiration of leaves of sugarbeet. Plant Physiol. 51:43-47.